



Fry, C. H., Chakrabarty, B., Hashitani, H., Andersson, K-E., McCloskey, K., Jabr, R. I., & Drake, M. J. (2019). New targets for overactive bladder-ICI-RS 2109. *Neurourology and Urodynamics*. <https://doi.org/10.1002/nau.24228>

Peer reviewed version

Link to published version (if available):
[10.1002/nau.24228](https://doi.org/10.1002/nau.24228)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Wiley at <https://onlinelibrary.wiley.com/doi/full/10.1002/nau.24228> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

New Targets for Overactive Bladder - ICI-RS 2019

CH Fry¹, B Chakrabarty¹, H Hashitani², K-E Andersson³, K McCloskey⁴, RI Jabr⁵, MJ Drake⁶

¹School of Physiology, Pharmacology & Neuroscience, University of Bristol, UK; ²Department of Cell Physiology, Nagoya City University, Japan; ³Institute of Laboratory Medicine, Lund University, Sweden and Institute for Regenerative Medicine, Wake Forest University School of Medicine, USA; ⁴School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, UK; ⁵Faculty of Health & Biomedical Sciences, University of Surrey, UK; ⁶Bristol Medical School, University of Bristol, UK.

Short title: Novel targets for OAB

Key words: Cyclic nucleotides, β 3-agonists, TRP channels, fibrosis, small conductance K⁺ channels

Correspondence:

CH Fry

School of Physiology, Pharmacology & Neuroscience,
University of Bristol,
University Walk
Bristol BS8 1TD, UK

Email: chris.fry@bristol.ac.uk

Word count.

3761

Abstract

Aims. To review evidence for novel drug targets that can manage overactive bladder symptoms. **Methods.** A think tank considered evidence from the literature and their own research experience to propose new drug targets in the urinary bladder to characterise their use to treat overactive bladder. **Results.** Five classes of agents or cellular pathways were considered. 1) Cyclic nucleotide-dependent (cAMP and cGMP) pathways that modulate ATP release from motor nerves and urothelium. 2) Novel targets for beta-3 agonists, including the bladder wall vasculature and *muscularis mucosa*. 3) Several TRP channels (TRPV₁, TRPV₄, TRPA₁, TRPM₄) and their modulators in affecting detrusor overactivity. 4) small conductance Ca²⁺-activated K⁺ channels and their influence on spontaneous contractions. 5) antifibrosis agents that act to modulate directly or indirectly the TGF-beta pathway - the canonical fibrosis pathway. **Conclusions.** Specificity of action remains a consideration if particular classes of agents can be considered for future development as receptors or pathways that mediate actions of the above potential agents are distributed among most organ systems. The tasks are to determine: more detail of the pathological changes that occur in the overactive bladder and; how specificity of potential drugs may be directed to bladder pathological changes. An important conclusion was that the storage, not the voiding, phase in the micturition cycle should be investigated and potential targets lie in the whole range of tissue in the bladder wall and not just detrusor.

232 words.

Introduction.

This article is a summary of a Think Tank to identify potential novel targets to minimise symptoms of overactive bladder (OAB) and to suggest some research strategies to achieve this goal. Symptoms arising from primary neurological pathologies were beyond the scope of this discussion. The current pharmaceutical options to manage OAB symptoms remain a triad of drug classes which are presumed to suppress directly detrusor contractions: anticholinergic agents; β 3-receptor agonists; and pre-synaptic nerve-muscle blockers such as botulinum toxin. However, increasing evidence questions their predominant site of action as detrusor smooth muscle and in consequence this has stimulated the search for other drug targets on a range of cell types in the wall of the bladder. The cause of OAB is probably multifactorial so that new drug candidates are continuously being explored^{1,2} and this discussion considered evidence for a range of different, potentially useful targets on the bladder wall including: detrusor muscle itself; excitatory motor nerves; the urothelium; the suburothelium and the extracellular space.

Modulators of cyclic nucleotides and ATP release

Cyclic nucleotides, such as cyclic AMP and cyclic GMP, are second messengers in a large range of biological processes so that intracellular pathways involving cyclic nucleotide turnover and signalling can be targeted to modulate their action. In particular, the effects of modulating cyclic nucleotide dependent pathways that mediate adenosine triphosphate (ATP) release from bladder wall tissues are of interest, as ATP is a functional neuromodulator in human OAB. Thus, manipulation of ATP release through pathways regulated by cyclic nucleotides offers a route for therapeutic management of OAB.

Adenylyl cyclase (AC) modulates the conversion of ATP to cAMP, and thus is a target for non-selective activators of G-protein-coupled stimulatory pathways, via an action on the α subunit. For example, activation of β 3-adrenoceptors utilises the prototypical transduction pathway for the G_s protein family, modulating AC activity and increasing the level of cAMP³. The β 3-adrenoceptor agonist, mirabegron, is used to treat OAB, and induces relaxes detrusor smooth muscle contractile activity *via* cAMP-dependent and -independent pathways⁴. β 3-adrenoceptor agonists, BRL37344 and CL316243, reduce nerve-mediated contractions, with a greater effect on the atropine-resistant component of nerve-mediated contractions in

comparison to the cholinergic component, and reduce P2X receptor-mediated contractions⁵. The AC activator, forskolin, also attenuates ATP-mediated responses; actions mirrored by activators of intermediates involved in downstream signalling of cAMP. These include 6-MB-cAMP (a protein kinase A (PKA) activator), and 007-AM the EPAC (Exchange Protein Activated by cAMP) EPAC activator. This demonstrates that β 3-adrenoceptor agonists reduce post-junctional ATP-dependent responses in detrusor smooth muscle to a greater extent than acetylcholine-activated responses via cAMP-dependent pathways⁵.

AC is also the target for the non-selective activators of the inhibitory G protein α subunit; for example, activation of adenosine A1 receptors utilises the prototypical transduction pathway for the G_i/G_o protein family, inhibiting AC activity and decreasing cAMP levels³. Adenosine reduces nerve-mediated contractions of human detrusor muscle more from patients with neuropathic detrusor overactivity (NDO) than those with stable bladders⁶. In NDO detrusor samples, there is a greater effect at lower frequencies, i.e. where nerve-mediated contractions have a greater dependence on ATP release, compared to higher frequencies. These effects are mimicked by the adenosine A1 receptor agonist, N6-cyclopentyladenosine (CPA)⁶. The proposed mechanism of action is that adenosine, metabolised from ATP at the neuromuscular junction via ectoATPases, acts at A1 receptors at the nerve terminal, resulting in inhibition of further neuronal ATP release from the efferent nerve. This generates a self-limiting response to excessive nerve-mediated ATP release⁶. Reduction of ectoATPase activity would reduce this negative feedback and enhance purinergic contractions. Adenosine also reduces distention-induced ATP release from rabbit urothelium or enhanced by the A1 receptor antagonist DPCPX or by adenosine deaminase, an enzyme that catalyses adenosine breakdown⁷. Thus, adenosine, produced by ATP hydrolysis, has a negative feedback effect on ATP release from both efferent nerve terminals and the urothelium *via* A1 receptor activation. The nitric oxide (NO)-sensitive soluble guanylyl cyclase (sGC) converts guanosine triphosphate to cGMP³. sGC is the target of endogenous NO, and sGC activators and modulators, such as BAY 41-2272 and BAY 60-2770, directly reduce bladder smooth muscle contractions^{8,9}. The sGC activator BAY 58-2667 reduces ATP release and nerve-mediated contractions from efferent nerve terminals in isolated detrusor preparations [Chakrabarty B, Drake MJ, Kanai AJ, Fry CH, unpublished data].

Phosphodiesterases (PDEs) catalyse the hydrolysis of CNTs, with some subtypes expressing an affinity for cAMP and cGMP, whilst others have a greater affinity for cAMP or cGMP³. PDE type-5 (PDE5) has a greater affinity for cGMP and sildenafil, a PDE5 inhibitor, elevates intracellular cGMP³ and in humans alleviates LUTS¹⁰. Sildenafil demonstrates a direct relaxant effect on human detrusor smooth muscle¹¹. Moreover, it also attenuates nerve-mediated contractions, with a greater effect at the lower frequency, atropine-resistant, ATP-dependent component of nerve-mediated contractions¹². Sildenafil also reduced nerve-mediated neuronal ATP release, and stretch-activated urothelial ATP release from isolated mucosal strips¹².

Targeting cyclic nucleotide-dependent pathways, or pathways involved in cyclic nucleotide turnover or signalling modulates the purinergic components of bladder contractions and urothelial ATP release. Targeting ATP release should attenuate pathological processes associated with enhanced ATP-dependent motor and sensory responses.

Targets of β 3-adrenoceptor agonists in the bladder?

Nerve-mediated and spontaneous contractions. Detrusor smooth muscle relaxation is proposed as a primary mechanism of β 3-adrenoceptor agonists to improve bladder storage dysfunction. However, relatively high concentrations are required to relax pre-contracted isolated detrusor strips, with IC₅₀ values of approximately 1 μ M¹³ and a similar potency is seen by reducing resting tone in human detrusor¹⁴. With mirabegron, currently the only clinically approved β 3-adrenoceptor agonist, the maximum plasma concentration of its therapeutic dose is lower than that required to relax isolated detrusor¹⁵. However, direct attenuation of voiding contractions would be physiologically undesirable as this may cause urinary retention. Thus, β 3-adrenoceptor agonists could act at additional sites on the bladder wall. The therapeutic effect of β 3-adrenoceptor agonists is based on a widely-accepted role of sympathetic nerves during the storage phase; noradrenaline release from sympathetic nerves binds to detrusor muscle β 3-adrenoceptors to reduce its contractility. However, sympathetic nerves to the bladder wall almost exclusively innervate blood vessels with very few fibers are distributed in the detrusor smooth muscle itself¹⁶. Thus, whilst sympathetic, nerve-mediated β -adrenergic detrusor relaxation is difficult to detect, it is possible that direct application of β -adrenergic agonists relaxes detrusor *via* 'extra-junctional' receptors¹⁷.

β 3-adrenoceptor agonists also reduce acetylcholine (ACh) release or cholinergic nerve-mediated detrusor contractions at clinically relevant concentrations¹⁸⁻²⁰; through a direct action

on receptors expressed on cholinergic nerves, or via release of adenosine from detrusor that subsequently reduces cholinergic neurotransmission via A1 receptor activation¹⁹. At present, different studies with human tissue show different β 3-adrenoceptor expression patterns on detrusor muscle or cholinergic nerves; some with an emphasis of localization on detrusor, but not cholinergic nerve fibres (e.g.¹⁹), and others suggesting almost exclusive localization on cholinergic, adrenergic or sensory peptidergic fibres, but none on detrusor smooth muscle itself (e.g.²⁰). There may also be species differences of localisation and magnitude of expression as Western blot assays showed localisation of β 3-adrenoceptor in human detrusor tissue but not in rat, that may in part be due to antibody affinity to the targets in rat and human tissue. It is evident that more data are required to characterize β 3-adrenoceptor location in bladder wall tissue from human and animals.

Enhanced spontaneous detrusor phasic contractions, or bladder micromotions, during the storage phase are critical in urinary urgency²¹ and β 3-adrenoceptor agonists may act more specifically to suppress aberrant spontaneous activity. The relevance of an action on spontaneous contractions is that they, in turn, profoundly influence afferent firing from the bladder wall. A mouse cystometric study showed that the magnitude and rate of change of spontaneous transient contractions had a 10-fold larger impact on afferent nerve firing compared with base-line changes²². Thus, attenuation of aberrant spontaneous detrusor contractions or their rate of change - but not baseline tension - may be sufficient to improve bladder storage function. These findings reveal two important research questions regarding β 3-adrenoceptor agonists: their relative potency to suppress spontaneous vs receptor-modulated contractions; and how they modulate spontaneous contractions.

Both ACh and ATP are spontaneously released from nerves *via* TTX-insensitive mechanisms^{23,24}. These may sum (in the case of ATP) to evoke excitatory junctional potentials and even detrusor action potentials. The role of β 3-adrenoceptor agonists to attenuate spontaneous release of excitatory transmitters has not been explored. Because purinergic transmission is more evident in pathological human bladders, attenuation of ATP release may be more relevant. Moreover, β 3-adrenoceptor agonists have more pronounced inhibitory effects on purinergic than cholinergic transmission⁵.

The role of the mucosa. Bladder mucosa contains several cellular elements that could be a target for β 3-adrenoceptor agonists including: urothelium, afferent nerves, interstitial cells and/or

fibroblasts, blood vessels and *muscularis mucosae*. As discussed above, attenuation of 'mechanosensitive' afferent nerve firing by β 3-adrenoceptor agonists may be a result of associated reduction of non-voiding spontaneous contractions. Although non-voiding contractions may originate from detrusor, the bladder mucosa also has contractile properties, in several species including human²⁵. A predominant contribution is from *muscularis mucosae*: it generates some ten-times more contractile strength compared to detrusor, when normalised to cross-section area, but only modestly responds to cholinergic stimulation²⁶. Thus, it may be suggested that contraction of the *muscularis mucosae* is important in the storage phase, but not the voiding phase. Moreover, the close proximity of *muscularis mucosae* to afferent nerves offers another 'mechanosensitive' mode of afferent activation. The action of β 3-adrenoceptor agonists on *muscularis mucosae* contraction is unknown but β 3-adrenoceptor expressing nerves are abundantly distributed in the suburothelium²⁰. It is of note that rat and mouse bladder lack a *muscularis mucosae*, and their mucosa is virtually non-contractile²⁵.

Effects on bladder wall blood vessels. The vasculature of the bladder forms an extensive suburothelial capillary plexus so that the mucosa receives generous blood supply, at least during the storage phase²⁷. Sympathetic nerves predominantly innervate bladder wall blood vessels and, in rat and mouse bladder, provide a functional innervation to suburothelial arterioles and venules²⁸. Neurally-released noradrenaline constricts venules via α -adrenoceptor activation, while causing vasodilatation via β -adrenoceptor stimulation. Thus, it may be proposed that β 3-adrenoceptors exert a therapeutic action by improving tissue perfusion²⁵. In arterioles, mirabegron (100 nM) suppresses nerve-evoked Ca^{2+} transients by about 30% - a concentration similar to plasma levels (H Hashitani, unpublished data). Blood flow distribution within the bladder wall is regulated by the microvasculature, functioning as an integrated unit²⁹. Thus, the expression pattern of β 3-adrenoceptors in different vascular segments and the response to β 3-adrenoceptor agonists in regulating local and global blood flow should be explored.

TRP channels

Several channels of the TRP family have a role in nociception and mechanosensory transduction in the lower urinary tract (LUT). A number of these channels, including TRPV₁, TRPV₄, TRPM₈, TRPA₁ and TRPM₄, have been suggested from animal studies to be able to treat OAB/DO, bladder underactivity and bladder pain disorders^{30,31}. OAB is a filling, rather than a voiding,

disorder and demonstrates altered afferent signaling patterns. This implies that modulation of pathways that determine bladder afferent activity can be effective therapies for OAB.

TRPV₁ channels. The function of TRPV₁ channels in normal human bladder activity remains unclear. However, the therapeutic benefit in neurogenic DO (NDO) of agonists that desensitise TRPV₁ channels (capsaicin, resiniferatoxin; when given intravesically) has been convincingly demonstrated. These agents are proposed to desensitise TRPV₁ channels and so inactivate afferent neurons.

Although there is no recent development of TRPV₁ agonists to manage OAB/DO, animal experiments in different models of DO have suggested that blockade of TRPV₁ receptors by small molecule compounds may have translational impact³². These agents have been developed for non-bladder problems and tested in human trials for safety but have not been used clinically to treat LUT dysfunction³¹. The first generation of TRPV₁ blockers have been associated with adverse effects such as hyperthermia and reduced sensation to noxious heat, and this has delayed development. However, despite these adverse effects, small molecule TRPV₁ channel antagonists deserve consideration for translation to future drugs to manage LUT.

TRPV₄ channels. TRPV₄ channels are present in urothelium and detrusor muscle cells and in urothelium are proposed to have important mechanosensory functions during bladder distension³². With cystitis rodent models, induced by cyclophosphamide, HC-067047, the selective TRPV₄ antagonist reduced the frequency of micturition and augmented functional bladder capacity³². With rats undergoing repeated external stresses, DO is induced by intravesical addition of TRPV₄ agonists and improved by intravesical administration of HC-067047. These data suggest TRPV₄ channels are a promising target for bladder overactivity disorders³³. However, there are no current data about the effects of HC-067047 on human lower urinary tract dysfunction.

TRPM₈ channels. TRPM₈ channels are expressed in bladder mucosa and their abundance is positively associated voiding frequency in samples from patients with idiopathic DO. This has led to the proposal that this channel subtype contributes to the pathophysiology of this disorder³⁴. In humans, treatment with a selective TRPM₈ antagonist, PF-05105679, reduced significantly pain from a cold pressor test, but had no effect on core temperature³⁵. However, with two subjects in his study there was an intolerable sensation of perioral heat, an adverse effect that has precluded further tests

TRPA₁ channels. These channels sense noxious stimuli in animal and human bladder. They are expressed in some capsaicin-sensitive sensory afferents and react as polymodal sensors to a number of extracellular or intracellular physical and chemical stimuli³¹. TRPA₁ channels have also been identified on urethral C-fibre afferents and urothelial cells as well as detrusor myocytes from human tissue. Furthermore, intravesical application of TRPA₁ channel activators initiate DO-like activity. Together it has been proposed that they have a role in modulating both efferent and afferent fibre activity in both the bladder and outflow tract³¹. There seems to be no information on the use of TRPA₁ antagonists in OAB/DO treatment in humans, but TRPA₁ antagonists for alleviation pain has been tested in preliminary trials³⁶.

TRPM₄ channels. These monovalent cation-selective channels are broadly expressed in several rodent and human tissues, including urothelium and detrusor. Furthermore, they are upregulated in the urothelium and detrusor of mice after spinal cord transection³⁷. Blockade of TRPM₄ channels in human detrusor myocytes with 9-phenanthrol reduced spontaneous inward current transients, associated with their hyperpolarisation and attenuation of spontaneous contractions in tissue strips³⁸. These studies suggest TRPM₄ channels but there are no published trials in humans.

Several TRP channel subtypes are found in lower urinary tract tissues and a number of animal studies lend weight to the possibility that channel modulation may have therapeutic value to manage LUT functional disorders. However, there are caveats to this approach: animal models of LUT dysfunction can be unreliable; and the efficacy of channel modulators in humans is unclear. However, they remain interesting potential targets for future development.

BK/SK K⁺ channels

Over several decades, the physiological relevance of detrusor K⁺ channels in bladder physiology has been recognised as having the potential to be harnessed to treat DO. A complement of K⁺ channels contribute to maintaining the resting membrane potential, as well as action potential repolarisation and after-hyperpolarization in detrusor smooth muscle cells³⁹. These include Ca²⁺-activated K⁺ channels with large or small unit ion conductance; BK and SK channels respectively. The hypothesis that K⁺ channel openers, particularly BK channel openers, could be developed to limit detrusor smooth muscle excitability and therefore treat overactivity, has been tested in bladder tissue and cells from humans and small animals.

BK and SK channel blockers, iberiotoxin and apamin respectively, enhance spontaneous contractions in human bladder strips, demonstrating channel functionality under normal conditions^{40,41}. Patch-clamp electrophysiology has characterised pharmacological and biophysical properties of BK currents in human detrusor myocytes from normal bladders⁴². However, neither iberiotoxin nor a BK channel opener, affected spontaneous contractions in bladder tissue from neurogenic bladders⁴² and PCR experiments indicated significant downregulation of the BK α gene, KCNMA1, in neurogenic bladder⁴¹. These reports are consistent with observations of bladder overactivity in mice lacking BK channels⁴³. It is therefore perhaps unsurprising that clinical trials of BK channel openers as putative drug treatments for detrusor overactivity have been unsuccessful⁴⁴. The possibility of restoring BK expression in neurogenic human detrusor smooth muscle cells via gene therapy has been promising in pre-clinical *in vivo* studies⁴⁵, has been trialled in human erectile dysfunction patients⁴⁶ and is being explored in clinical trials with intravesical application of BK units⁴⁷. The results of these clinical trials on BK channel modulation in OAB patients have not yet been fully reported.

The observation that blockade of SK channels enhanced spontaneous contractions in neurogenic bladder tissue⁴² suggests that SK channel openers could also be developed to treat bladder overactivity. The SK channel opener SKA-31 decreased spontaneous contractions in normal human bladder detrusor strips⁴⁸. However, its additional reduction of nerve-evoked contractions also highlighted the fact that SK openers to treat overactive bladder may adversely impact voiding contractions leading to urinary retention. Further research is needed in this area.

Anti-fibrosis strategies

Bladder wall fibrosis and overactive bladder symptoms. Many bladder pathologies that demonstrate OAB symptoms also shows replacement of detrusor smooth muscle with connective tissue (fibrosis) that consists of collagens and elastin embedded in a ground substance of proteoglycans⁴⁹. This apparently counter-intuitive observation may be explained in several ways: ECM is generally stiffer than muscle tissue so that bladder wall compliance is reduced, with sensory thresholds achieved at lower filling volumes; detrusor spontaneous contractions arise when nerve-mediated responses are suppressed⁵⁰; and the increased density of collagen-producing cells, such as myofibroblasts and interstitial cells, themselves generate

irregular propagating electrical signals⁵¹. Reduction of ECM deposition will therefore be a useful target to restore normal bladder function.

TGF- β and fibrosis. TGF- β is a polypeptide cytokine that belongs to a transforming growth factor superfamily including three mammalian isoforms, TGF- β 1 to - β 3. They are released by many cells in an inactive form, but when activated by several extracellular proteinases they bind to receptors (TGFR1,2) that initiate intracellular signalling pathways leading to fibrosis⁵². A canonical pathway consists of: phosphorylation of associated Smad proteins (Smad2 and Smad3) and binding to Smad4; Smad3 binding to gene promoters that induce transcription of fibrosis products, tissue inhibitor of matrix metalloproteinase (MMP) and transformation of fibroblasts and epithelial cells to collagen-producing myofibroblasts. In this pathway Smad2 and Smad4 act as regulators of Smad3. TGFR-Smad signalling can in turn interact with other potentially fibrosis-inducing pathways including Wnt/ β -catenin, that is present at least in the neonatal bladder⁵³.

Modulation of the of TGF- β pathway. Attenuation of the above pathway should in principle reduce fibrosis and two examples will exemplify this approach. Bone morphogenic protein-7 (BMP-7) is part of the TGF- β superfamily that on binding to its receptor also induces phosphorylation of Smad proteins – Smad1,5,8. These require the same chaperone, Smad4, to translocate to the nucleus, in this case to initiate anti-fibrotic pathways. Thus, not only will this pathway be initiated by BMP-7 but its sequestration of Smad4 will also reduce the fibrotic TGF- β pathway. Proof-of-principle has been demonstrated in several tissues, including liver and kidney⁵⁴, although there are no data regarding bladder fibrosis.

Peroxisome proliferator-activated receptors (PPAR) are nuclear transcription factors with antifibrotic effects. There are three known subtypes (α , β and γ) and PPAR γ has received most attention as it is the target for oral antidiabetic drugs, thiazolidinediones. The antifibrotic effects of PPAR agonists on many tissues has long been known and is largely through inhibition of the TGF- β pathway, or the Wnt pathway, at a number of sites⁵⁵. However, experience with bladder fibrosis is lacking and as with BMP-7 these offer potential antifibrotic strategies to be pursued.

Antifibrotic actions of relaxin and cGMP. The insulin superfamily in humans consists of insulin, insulin-like growth factors and three relaxin subtypes (H1-3) and several insulin-like peptides.

Relaxin peptides are agonists for relaxin family peptide (RXFP) G-protein coupled receptors, with the most studied interaction between H2 and RXFP1⁵⁶. Antifibrotic actions have been recorded in the lung, kidney, liver, portal vein and heart, which has been translated into successful clinical trials, as well as in rat bladder subjected to X-ray irradiation⁵⁷. With the rat bladder, cystometry showed reversal of a phenotype showing a low compliance, overflow incontinence pattern with spontaneous contractions to a normal high compliance bladder with transient voiding contractions. The return of voiding contractions was mirrored by enhanced in vitro detrusor contractions and enhanced expression of L-type Ca^{2+} channel units demonstrating a multi-faceted action of relaxin, as observed in other organs such as the kidney⁵⁸. The antifibrotic action of relaxin, in part, is through modulation of the TGF- β pathway. RXFP1 activation by H2 eventually increases intracellular cGMP, via ERK1 phosphorylation, nitric oxide synthase activation and NO-dependent soluble guanylate cyclase (sGC) activation⁵⁹: cGMP itself prevents phosphorylation of the Smad2,3 complex.

More immediate ways to increase intracellular cGMP are through attenuation of its breakdown through inhibition of phosphodiesterase5 (PDE5) or direct action of sGC. PDE5 inhibitors, including, sildenafil and tadalafil have been extensively investigated in the genito-urinary tract as direct or indirect smooth muscle relaxants. Several sGC activators, such as riociguat and cinaciguat, have also been developed initially to minimise cardiovascular problems. The potential usefulness of PDE5 inhibitors to reduce fibrosis in the genito-urinary tract has been proposed⁶⁰ with initial studies in prostate and corpus cavernosal tissue have been. However, to our knowledge no systematic investigations have targeted the bladder. Similarly, sGC activators have anti-fibrotic actions in many experimental models of renal and cardiovascular diseases⁶¹ and direct application to the bladder is awaited.

Other targets

This review has summarised the discussion held at ICI-RS 2019 and so is not comprehensive of new advances that have identified potential drug targets to mitigate the effects of OAB. However, it is important to recognise additional advances made in identifying LUT-selective targets, for example in areas of purinergic signalling and modulation of other K^+ channels. OAB is associated with increased efferent nerve and urothelial purinergic signalling with one potential target at P2X₃ receptors on afferent nerves. The P2X₃ receptor antagonist AF-219 has been shown to attenuate afferent signalling: it is currently under clinical trials for chronic cough

syndromes⁶², proposed for hypertension⁶³ and reported for bladder pain syndrome⁶⁴. An increase of the conductance of K⁺ channels is also an attractive target to reduce overactive bladder contraction. However, a major obstacle is an off-target effect of relaxing vascular smooth muscle and so generating hypotension. Recent advances that bladder-specific isoforms of Kv7 channels may be identified and their further development is awaited with interest^{65,66}.

Conclusion

OAB medications can be administered intravesically, but the value of alternative oral drugs is clearly preferable. However, the presence of targets in the bladder may well be matched by expression in other crucial organ systems, such as the cardiovascular and neurological systems, and thus far has limited development of oral compounds that affect TRP and K⁺ channels. Antimuscarinic medications are accepted as the severity of their systemic influences is often manageable and β 3 agonists and PDE5 inhibitors have a low chance of substantial systemic adverse effects. Thus, deeper insight into their mechanisms of action is valuable for two reasons: firstly, understanding how they work will help optimise their use (e.g. is there a particular phenotype of presentation that would predict a good response); secondly, drugs targeting downstream elements of intracellular pathways could be more efficacious. For example, soluble GC activators are interesting as this enzyme is a downstream target of PDE5 inhibitors. Moreover, recent work has recognised that modulation of the storage phase, rather than the voiding phase, is important to manage OAB symptoms, even with current agents. In this context there should be a greater emphasis on future research describing how current and potentially useful agents have direct or indirect actions of afferent nerve activity. Furthermore, how such information is processed in the central nervous system will also be crucial to understand, including if these agents have additional central actions rather than purely on the bladder wall.

References

- 1 Peyronnet B, Mironska E, Chapple C, Cardozo L, Oelke M, Dmochowski R et al. A comprehensive review of overactive bladder. pathophysiology: on the way to tailored treatment. *Eur Urol* 2019; 75: 988-1000.
- 2 Chen LC, Kuo HC. Pathophysiology of refractory overactive bladder. *Low Urin Tract Symptoms*. 2019 Mar 22. doi: 10.1111/luts.12262.
- 3 Alexander SP, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E et al. The concise guide to PHARMACOLOGY 2017/18: Enzymes. *Br J Pharmacol* 2017; 174(Suppl 1): S272-S359.
- 4 Maki T, Kajioka S, Itsumi M, Kareman E, Lee K, Shiota M et al. Mirabegron induces relaxant effects via cAMP signaling dependent and -independent pathways in detrusor smooth muscle. *Low Urin Tract Symptoms* 2019; 11: O209-O217.
- 5 Fong Z, Griffin CS, Hollywood MA, Thornbury KD, Sergeant GP. β 3-adrenoceptor agonists inhibit purinergic receptor-mediated contractions of the murine detrusor. *Am J Physiol Cell Physiol* 2019; 317: C131-C142.
- 6 Pakzad M, Ikeda Y, McCarthy C, Kitney DG, Jabr RI, Fry CH. Contractile effects and receptor analysis of adenosine-receptors in human detrusor muscle from stable and neuropathic bladders. *Naunyn Schmiedebergs Arch Pharmacol* 2016; 389: 921-929.
- 7 Dunning-Davies BM, Fry CH, Mansour D, Ferguson DR. The regulation of ATP release from the urothelium by adenosine and transepithelial potential. *BJU Int* 2013; 111: 505-513.
- 8 Füllhase C, Hennenberg M, Sandner P, Strittmatter F, Niedworok C, Bauer RM et al. Reduction of obstruction related bladder overactivity by the guanylyl cyclase modulators BAY 41-2272 and BAY 60-2770 alone or in combination with a phosphodiesterase type 5 inhibitor. *Neurourol Urodyn* 2015; 34: 787-793.
- 9 Leiria LO, Silva FH, Davel AP, Alexandre EC, Calixto MC, De Nucci G et al. The soluble guanylyl cyclase activator BAY 60-2770 ameliorates overactive bladder in obese mice. *J Urol* 2014; 191: 539-547.
- 10 McVary KT, Monnig W, Camps JL, Young JM, Tseng LJ, van den Enge G. Sildenafil citrate improves erectile function and urinary symptoms in men with erectile dysfunction and lower urinary tract symptoms associated with benign prostatic hyperplasia: A randomized, double-blind trial. *J Urol* 2007; 177: 1071-1077.
- 11 Oger S, Behr-Roussel D, Gorny D, Lebret T, Validire P, Cathelineau X et al. Signalling pathways involved in sildenafil-induced relaxation of human bladder dome smooth muscle. *Br J Pharmacol* 2010; 160: 1135-1143.
- 12 Chakrabarty B, Ito H, Ximenes M, Nishikawa N, Vahabi B, Kanai AJ et al. Influence of sildenafil on the purinergic components of nerve-mediated and urothelial ATP release from the bladder of normal and spinal cord injured mice. *Br J Pharmacol* 2019; 176: 2227-2237.
- 13 Takeda M, Obara K, Mizusawa T, Tomita Y, Arai K, Tsutsui T et al. Evidence for β 3-adrenoceptor subtypes in relaxation of the human urinary bladder detrusor: analysis by molecular biological and pharmacological methods. *J Pharmacol Exp Ther*. 1999; 288: 1367-73.
- 14 Igawa Y, Yamazaki Y, Takeda H, Kaidoh K, Akahane M, Ajisawa Y et al. Relaxant effects of isoproterenol and selective beta3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. *J Urol*. 2001 ; 165:240-4.
- 15 Michel MC, Korstanje C. β 3-Adrenoceptor agonists for overactive bladder syndrome: role of translational pharmacology in a repositioning clinical drug development project. *Pharmacol Ther*. 2016; 159: 66–82.

- 16 Gosling JA, Dixon JS, Jen PY. The distribution of noradrenergic nerves in the human lower urinary tract. A review. *Eur Urol.* 1999; 36 Suppl 1:23-30.
- 17 Persson K, Andersson KE. Nitric oxide and relaxation of pig lower urinary tract. *Br J Pharmacol.* 1992; 106:416-22.
- 18 Agostino G, Maria Condino A, Calvi P. Involvement of β 3-adrenoceptors in the inhibitory control of cholinergic activity in human bladder: Direct evidence by [(3)H]-acetylcholine release experiments in the isolated detrusor. *Eur J Pharmacol.* 2015; 758:115-22.
- 19 Silva I, Costa AF, Moreira S, Ferreirinha F, Magalhães-Cardoso MT, Calejo I et al. Inhibition of cholinergic neurotransmission by β 3-adrenoceptors depends on adenosine release and A1-receptor activation in human and rat urinary bladders. *Am J Physiol Renal Physiol.* 2017; 313: F388-F403.
- 20 Coelho A, Antunes-Lopes T, Gillespie J, Cruz F. β -3 adrenergic receptor is expressed in acetylcholine-containing nerve fibers of the human urinary bladder: An immunohistochemical study. *Neurourology and Urodynamics.* 2017; 36: 972–1980.
- 21 Drake MJ, Harvey IJ, Gillespie JI, Van Duyl WA. Localized contractions in the normal human bladder and in urinary urgency. *BJU International.* 2005; 95: 1002–1005.
- 22 Heppner TJ, Tykocki NR, Hill-Eubanks D, Nelson MT. Transient contractions of urinary bladder smooth muscle are drivers of afferent nerve activity during filling. *J Gen Physiol.* 2016; 147:323–335.
- 23 Zagorodnyuk VP, Gregory S, Costa M, Brookes SJ, Tramontana M, Giuliani S, Maggi CA. Spontaneous release of acetylcholine from autonomic nerves in the bladder. *Br J Pharmacol.* 2009; 157:607-19.
- 24 Hayase M, Hashitani H, Kohri K, Suzuki H. Role of K^+ channels in regulating spontaneous activity in detrusor smooth muscle in situ in the mouse bladder. *J Urol.* 2009; 181:2355-65.
- 25 Mitsui R, Lee K, Uchiyama A, Hayakawa S, Kinoshita F, Kajioka S et al. Contractile elements and their sympathetic regulations in the pig urinary bladder: A species and regional comparative study. *Cell Tissue Res.* In the Press
- 26 Lee K, Mitsui R, Kajioka S, Naito S, Hashitani H. Role of PTHrP and sensory nerve peptides in regulating contractility of muscularis mucosae and detrusor smooth muscle in the guinea pig bladder. *J Urol.* 2016; 196:1287–1294.
- 27 Brading AF, Greenland JE, Mills IW, McMurray G, Symes S. Blood supply to the bladder during filling. *Scand J Urol Nephrol Suppl.* 1999; 201:25-31.
- 28 Shimizu Y, Mochizuki S, Mitsui R, Hashitani H. Neurohumoral regulation of spontaneous constrictions in suburothelial venules of the rat urinary bladder. *Vascul Pharmacol.* 2014; 60:84-94.
- 29 Hashitani H, Mitsui R, Miwa-Nishimura K, Lam M. Role of capillary pericytes in the integration of spontaneous Ca^{2+} transients in the suburothelial microvasculature in situ of the mouse bladder. *J Physiol.* 2018; 596: 3531-3552.
- 30 Andersson KE. Potential future pharmacological treatment of bladder dysfunction. *Basic Clin Pharmacol Toxicol* 2016; 119 Suppl 3: 75-85.
- 31 Andersson KE. TRP channels as lower urinary tract sensory targets. *Med Sci* 2019; 7: pii: E67.
- 32 Everaerts W, Zhen X, Ghosh D, Vriens J, Gevaert T, Gilbert J P et al. Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. *Proc Natl Acad Sci U S A* 2010; 107: 19084-19089.

- 33 Merrill L, Vizzard MA. Intravesical TRPV4 blockade reduces repeated variate stress-induced bladder dysfunction by increasing bladder capacity and decreasing voiding frequency in male rats. *Am J Physiol Regul Integr Comp Physiol* 2014; 307: R471-R480.
- 34 Mukerji G, Yiangou Y, Corcoran SL, Selmer IS, Smith GD, Benham CD et al. Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. *BMC Urol* 2006 Mar 6;6:6.
- 35 Winchester WJ, Gore K, Glatt S, Petit W, Gardiner JC, Conlon K et al. Inhibition of TRPM8 channels reduces pain in the cold pressor test in humans. *J Pharmacol Exp Ther* 2014; 351: 259-269.
- 36 Koivisto A, Jalava N, Bratty R, Pertovaara A. TRPA1 Antagonists for Pain Relief. *Pharmaceuticals* 2018; 11: pii: E117.
- 37 Hristov KL, Smith AC, Parajuli SP, Malysz J, Rovner ES, Petkov GV. Novel regulatory mechanism in human urinary bladder: central role of transient receptor potential melastatin 4 channels in detrusor smooth muscle function. *Am J Physiol Cell Physiol* 2016; 310: C600-C611.
- 38 Kullmann FA, Beckel JM, McDonnell B, Gauthier C, Lynn AM, Wolf-Johnston A et al. Involvement of TRPM4 in detrusor overactivity following spinal cord transection in mice. *Naunyn Schmiedebergs Arch Pharmacol* 2018; 391: 1191-1202.
- 39 Petkov GV. Role of potassium ion channels in detrusor smooth muscle function and dysfunction. *Nat Rev Urol* 2011; 9: 30-40.
- 40 Darblade B, Behr-Roussel D, Oger S, Hieble JP, Lebret T, Gorny D et al. Effects of potassium channel modulators on human detrusor smooth muscle myogenic phasic contractile activity: potential therapeutic targets for overactive bladder. *Urology* 2006; 68: 442-448.
- 41 Hristov KL, Chen M, Kellett WF, Rovner ES, Petkov GV. Large-conductance voltage- and Ca^{2+} -activated K^+ channels regulate human detrusor smooth muscle function. *Am J Physiol, Cell Physiol* 2011; 301: C903-C912.
- 42 Oger S, Behr-Roussel D, Gorny D, Bernabe J, Comperat E, Chartier-Kastler E et al. Effects of potassium channel modulators on myogenic spontaneous phasic contractile activity in human detrusor from neurogenic patients. *BJU Int* 2011; 108: 604-611.
- 43 Meredith AL, Thorneloe KS, Werner ME, Nelson MT, Aldrich RW. Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+} -activated K^+ channel. *J Biol Chem* 2004; 279: 36746-36752.
- 44 Bentzen BH, Olesen SP, Ronn LC, Grunnet M. BK channel activators and their therapeutic perspectives. *Front Physiol* 2014; 5: 389.
- 45 Christ GJ, Day NS, Day M, Santizo C, Zhao W, Sclafani T et al. Bladder injection of "naked" hSlo/pcDNA3 ameliorates detrusor hyperactivity in obstructed rats in vivo. *Am J Physiol Regul Integr Comp Physiol* 2001; 281: R1699-R1709.
- 46 Melman A, Davies KP. Gene therapy in the management of erectile dysfunction (ED): past present and future. *ScientificWorldJournal*. 2009; 9: 846-854.
- 47 Petkov GV. Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. *Am J Physiol; Regul, Integ Comp Physiol* 2014; 307: R571-R584.
- 48 Soder RP, Parajuli SP, Hristov KL, Rovner ES, Petkov GV. SK channel-selective opening by SKA-31 induces hyperpolarization and decreases contractility in human urinary bladder smooth muscle. *Am J Physiol; Regul, Integ Comp Physiol* 2013; 304: R155-R163.
- 49 Fry CH, Kitney DG, Paniker J, Drake MJ, Kanai A, Andersson KE. Fibrosis and the bladder, implications for function ICI-RS 2017. *Neurourol Urodyn* 2018; 37: S7-S12.

- 50 Fry CH, McCloskey KD. Spontaneous Activity and the Urinary Bladder. *Adv Exp Med Biol* 2019; 1124: 121-147.
- 51 McCloskey K. Bladder interstitial cells: an updated review of current knowledge. *Acta Physiol* 2013; 207: 7-15.
- 52 Tang PM-K, Zhang Y-Y, Mak TS-K, Tang PC-T, Huang X-R, Lan H-Y. Transforming growth factor- β signalling in renal fibrosis: from Smads to non-coding RNAs. *J Physiol* 2018; 596: 3493-3503.
- 53 Johal NS, Arthurs C, Cuckow P, Cao K, Wood DN, Ahmed A, Fry CH. Functional, histological and molecular characteristics of human exstrophy detrusor. *J Pediatr Urol* 2019; 15: 154.e1-154.e9.
- 54 Li RX, Yiu WH, Tang SC-W. Role of bone morphogenetic protein-7 in renal fibrosis. *Front Physiol* 2015; 6: 114.
- 55 Dantas AT, Pereira MC, de Melo Rego MJ, da Rocha LF Jr, Pitta Ida R, Marques CD et al. The role of PPAR-gamma in systemic sclerosis. *PPAR Res* 2015; 1015: 124624.
- 56 Patil NA, Rosengren KJ, Separovic F, Wade JD, Bathgate RAD, Hossain MA. Relaxin family peptides: structure-activity relationship studies. *Br J Pharmacol* 2017; 174: 950-961.
- 57 Ikeda Y, Zabbarova IV, Birder LA, Wipf P, Getchell SE, Tyagi P et al. Relaxin-2 therapy reverses radiation-induced fibrosis and restores bladder function in mice. *Neurourol Urodyn* 2018; 37: 2441-2451.
- 58 Conrad KP, Davison JM. The renal circulation in normal pregnancy and preeclampsia: is there a place for relaxin? *Am J Physiol Renal Physiol* 2014; 306: F1121-1135.
- 59 Samuel CS, Royce SG, Hewitson TD, Denton KM, Cooney TE, Bennett RG. Anti-fibrotic actions of relaxin. *Br J Pharmacol* 2017; 174: 962-976.
- 60 Bushman WA, Jerde TJ. The role of prostate inflammation and fibrosis in lower urinary tract symptoms. *Am J Physiol, Renal Physiol* 2016; 311: F817-F821.
- 61 Stasch JP, Schlossmann J, Hocher B. Renal effects of soluble guanylate cyclase stimulators and activators: A review of the preclinical evidence. *Curr Opin Pharmacol* 2015; 21: 95-104.
- 62 Abdulqawi R, Dockry R, Holt K, Layton G, McCarthy BG, Ford AP, Smith JA. P2X₃ receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 2015; 385: 1198-1205.
- 63 Pijacka W, Moraes DJ, Ratcliffe LE, Nightingale AK, Hart EC, da Silva MP *et al.* Purinergic receptors in the carotid body as a new drug target for controlling hypertension. *Nature Med* 2016; 22: 1151-1159.
- 64 Moldwin R, Kitt M, Mangel J, Beyer R, Hanno P, Butera P *et al.* A phase 2 study in women with interstitial cystitis/bladder pain syndrome (IC/BPS) of the novel P2X₃ antagonist AF-219. International Continence society meeting, 2015, abstr 23.
- 65 Provence A, Malysz J, Petkov GV. The novel KV7.2/KV7.3 channel opener ICA-069673 reveals subtype-specific functional roles in guinea pig detrusor smooth muscle excitability and contractility. *J Pharmacol Exp Ther* 2015; 354: 290-301.
- 66 Bientinesi R, Mancuso C, Martire M, Bassi PF, Sacco E, Currò D. Kv7 channels in the human detrusor: channel modulator effects and gene and protein expression. *Naunyn Schmied Arch Pharmacol* 2017; 390: 127-137.